With the author's compliments.

(b)

ATMOSPHERIC LIFE GERMS.

BY

W. NOEL HARTLEY, F.C.S.

Reprinted from the Quarterly Journal of Science, *April*, 1873.

LONDON.

1873.

ATMOSPHERIC LIFE GERMS.

BY

W. NOEL HARTLEY, F.C.S.

Reprinted from the QUARTERLY JOURNAL OF SCIENCE, *April*, 1873.

LONDON.

1873.

ATMOSPHERIC LIFE GERMS.

LORD Bacon in the "Novum Organum" (Book II., Aphorism 13), says, "All putrefaction exhibits some slight degree of heat, though not enough to be perceptible to the touch: for neither the substances which by putrefaction are converted into animalculæ, as flesh and cheese, nor rotten wood which shines in the dark, are warm to the touch." He thus gives as a definition of spontaneous generation the conversion of substances, such as flesh and cheese, into animalculæ. The joke of Dr. Johnson on Tom Davies, a bankrupt bookseller, who took to authorship, that he was "an author generated by the corruption of a bookseller," is evidently a hint as to his connection with Grub Street through an allusion to the popular belief.

The first recorded facts undermining the old belief in "spontaneous generation," were those of Redi, published in 1638, leading to the first exact experiments in closed vessels of Needham in 1745, and of Spallanzani in 1765; the experiments with air purified by heating of Schwann, and with air passed through oil of vitriol of Schultze in 1837; the proof that the solid particles of yeast alone can cause fermentation by Helmholtz in 1844; Schrœder and Dusch's experiments with air filtered through cotton-wool in 1854; and the repetition of the foregoing and complete investigation of the subject by Pasteur in 1862. The object of this paper is to make these last experiments more widely known; unfortunately they must be stripped of detail, and thereby robbed of much of their strength of argument. Few persons are familiar with the mode of experimenting, the facts observed, and the remarkable chain of evidence afforded by these most carefully-executed, most complete, and therefore most trustworthy, experiments.

Pasteur's Microscopic Examination of the Solid Particles Diffused in the Atmosphere.

The question which Pasteur first set himself to answer was, Is it possible to gain an approximate idea of the relation a volume of ordinary air bears to the number of germs that the air may contain? Let us see what means were taken to determine the number and the nature of floating particles diffused in the air.

By means of a water aspirator air was drawn from a quiet street, and also from the gardens of the Ecole Normale, in Paris, at some distance from the ground, through

a tube containing a plug not of cotton-wool, as in the experiments of Schrœder, but of soluble pyroxyline, such as is used for making collodion. The amount of air aspirated in a given time was accurately measured, and after a sufficient interval the soluble cotton plug was removed and treated with its solvents, alcohol and ether. After allowing the dust to subside in a tube, the collodion was syphoned off, and more alcohol and ether added to effect the perfect removal of the collodion. The completely-washed dust was placed on a microscope slip and examined in a drop of water. By ordinary methods the action of different reagents, such as iodine water, potash, sulphuric acid, and colouring matters on the particles was tried. This process disclosed the fact that there is in ordinary air a variable number of corpuscles, ranging in size from extreme minuteness up to the diameters of 0·01 m.m. to 0·015 m.m.; some translucent particles of a regular shape so closely resemble the spores of the most common fungi that the most able microscopist could see no difference in them. The corpuscles were evidently organised, resembling completely the germs of the lowest organisms, and so diverse in size and structure as to belong without doubt to very various species. The soluble cotton used was previously tested and found to contain no residue insoluble in alcohol and ether beyond a fibre or two. By exposing a plug of pyroxyline for twenty-four hours to a current of air passing at the rate of a litre the minute after a succession of fine spring days, it was found that many myriads of organised corpuscles were collected.

Experiments with Heated Air.

Although it appears there are in air organised corpuscles in great numbers which are indistinguishable from the germs of the lowest organisms, is it really a fact that amongst these there are particles capable of germination? This interesting question was answered in a conclusive manner. Firstly, the facts announced by Schwann were firmly established, although they had previously been attacked by Mantegazza, Joly and Musset, and Pouchet. The solution, sealed up in flasks, was one extremely liable to change; its composition was—

Water	100 parts.
Sugar	10 ,,
Albumenoid and mineral matters from yeast	0·2 to 0·7 parts.

Boiled for two or three minutes, and then placed in contact

with air previously heated to redness, not a single doubtful result was obtained, although repeated at least fifty times; not a single trace of any organised production was seen even after eighteen months, keeping at a temperature of 25° to 30° C.; while, if the liquid be left to ordinary air for a day or two, it never fails to become filled with bacteria or vibriones, or covered with mould. The experiment of Schwann applied to this sugar solution is, therefore, of irreproachable exactitude. Schwann, however, did not always succeed so well as he wished, and the experience of Mantegazza and Pouchet was at variance with his general conclusions; even Pasteur himself in some experiments failed to preserve his liquids. These are the particular instances:—Five flasks of 250 c.c. capacity, containing 80 c.c. of the sugar solution, were boiled, and during ebullition sealed up. The points were broken under mercury, and pure gases in all cases but one let into the flasks. Organisms were found in every case after four days. In all these experiments, as in those likewise of Schwann, which were contrary to the result of his first experiment with extract of meat, it was the mercury that introduced the germs. In making such experiments with a mercury trough, preservation of the liquid will not always succeed, even if it succeeds sometimes. If the sugar solution be replaced by milk and treated by either of the methods above described, the milk putrefies. These results, so different and contradictory, find a natural explanation further on, but so far they are facts of a troublesome nature.

Germination of the Dust which exists suspended in the Air, in Liquids suitable to the Development of the Lowest Organisms.

The facts ascertained so far are:—

1. That there exist suspended in the air organised corpuscles exactly like the germs of the lowest organisms.

2. That sugar solutions with the liquor from beer yeast, a fluid extremely alterable in ordinary air, remains unchanged and limpid, without even giving rise to infusoria or fungi, when left in contact with air previously heated.

The question now arises, how is it possible to sow an albuminous sugar solution with germs collected by means of pyroxyline in the manner already described?

Taking a flask containing such a sugar solution kept at 25° to 30° C. for one or two months unchanged, in contact with previously heated air, the sealed-up end is connected by means of a caoutchouc tube with one part of a T tube, while another is in connection with an air-pump, and a

third with a platinum tube heated to redness. Between the T tube, however, and the flask is a wide tube containing a very narrow one within it, holding a plug of gun-cotton, through which a large volume of air has been passed. The tap in connection with the heated platinum tube was closed, and the one in connection with the air-pump opened ; after exhausting air was admitted through the red-hot platinum, the tap was closed, and the air again pumped out, fresh air being admitted through the heated tube ; this was repeated three or four times. The stop-cocks were then closed, and the sealed beak of the flask was broken within the india-rubber connection ; the plug of gun-cotton was shaken into the liquid, after which the flask was sealed up again. All experiments so performed resulted in the liquid, after three or four days' exposure to a temperature of 25° to 30° C., decomposing, and being found to contain bacteria, vibriones, and fungi, just exactly like those in flasks exposed to ordinary air. There was no difference in the length of time requisite for the change, the forms of life occurring, or the nature of the change resulting in flasks so treated, and those with the same liquid exposed to common atmospheric air. These experiments can scarcely be surpassed for beauty in their arrangement, or for the importance and clearness of the evidence they afford. Yet thinking that it might be objected that the gun-cotton had given rise to the changes produced, Pasteur made use of plugs of asbestos, and found a like result ; but when the plugs of asbestos were heated red-hot previous to being put into the flasks, the liquids remained unchanged in every case, and so constantly and with such perfect exactitude after an immense number of trials did the results remain the same, that the experimenter himself was astonished.

Extension of previous results to other very alterable Liquids—Urine, Milk, and Albuminous Sugar Solution mixed with Carbonate of Lime.

The facility with which urine exposed to the air becomes altered, and the change which takes place is well known. It becomes turbid and alkaline, sometimes filled with bacteria, or covered with patches of mucor or *Penicillium glaucum.* Often there is formed, when the temperature is not higher than 15° C., a pellicle consisting of a remarkable mucor closely resembling *torula*, but which is believed by Pasteur to be a different species. It consists of transparent cells, often without a nucleus, and considerably smaller than the cells of beer-yeast. There is also present in urine, when

alkaline from the carbonate of ammonia resulting from the changed urea, a peculiar fungus in necklace-like groups, and this organism Pasteur is fully persuaded is the cause of urea being converted into carbonate of ammonia. An interesting observation was made with regard to the turbidity of liquids, which generally is the first sign of alteration; this is caused not merely by the presence of minute organisms, such as bacteria, but by their movements in the liquid; for when they are dead they settle to the bottom of the vessel, and the liquid becomes clear again. Many flasks of urine were treated in the manner already described —that is to say, they were boiled, and heated air was admitted to them. After preservation for months at 25° to 30° C. without change, plugs of asbestos through which air had been drawn were introduced; and then in cases where the liquid was alkaline, strings of this peculiar fungus were found invariably, and crystals of ammonio-magnesian phosphate were deposited. It was observed that *Bacterium termo* appears in a liquid before any other organism. This infusorium is so small that it would be impossible to distinguish its germ; but even if the appearance of its germ were known it would be still less possible to recognise it among the various particles of organised dust collected from suspension in the atmosphere.

In experimenting with milk boiled in flasks and exposed to heated air, it was found that generally in from eight to ten days, but in one case after so long a time as a month, the milk was found to be curdled. Microscopic examination showed that the whey was filled with vibriones, often of the species *Vibrio lineola*, and bacteria. The air of the flasks showed that the oxygen was replaced by carbonic acid; yet swarms of these vibriones were living in an atmosphere without oxygen. The most important observation which leads to an explanation of the extraordinary behaviour of milk in these experiments, is the fact that no mucor, torula, or penicillium—nothing but bacteria or vibriones—were found in the liquid. The obvious conclusion is, that these organisms or their germs are not destroyed by a temperature of 100° C. when the heated liquid which serves to develop them enjoys certain properties. To test this supposition, the milk was boiled under pressure, so that the temperature was raised during ebullition to 110° C., and then heated air was admitted, of course at the usual atmospheric pressure; flasks treated in this way were kept an indefinite period without the production of any life whatever. The milk preserved its flavour, its odour, and all its

properties. Sometimes a slight oxidation of fatty matter took place, as could only be expected in such a considerable body of air; this was proved by an analysis of the air. In such cases the milk had a slightly suety taste. But what condition prevents the development of vibriones in sugar solutions and urine when heated to 100° C.? It is the fact that they contain a trace of acid. Milk is an alkaline liquid. If a liquid of the following composition:—

Sugar	10 grms.
Yeast water	100 c.c.
Carbonate of lime . . .	1 grm.

be boiled in flasks at 100° C., filled with heated air and sealed up and left to itself at 25° to 30°, in from two to four days it becomes turbid from vibriones, which have a very lively motion. It was found that a species of mucor after a time covered the surface of the liquid. It seems, therefore, that under these particular conditions, that the germs of this cryptogam had resisted the temperature of boiling water. An important confirmation of these experiments regarding the failure of a temperature of 100° C. to destroy certain germs here follows. Milk which had been preserved some months had a plug of asbestos presumably containing germs introduced into it by the manner already described; it was sealed up, and the flask was then plunged into boiling water; in eight days bacteria and vibriones were found in swarms. It was further discovered that 108° was too low a temperature to effect the preservation of these liquids.

It cannot be too forcibly impressed on the reader by what means and with what success Pasteur demonstrated the fact of myriads of organisms occupying comparatively small volumes of air. This is a point to which his detractors have willingly made themselves blind; they tell us the organisms are few in number without any experimental proof; while, on the other hand, Dr. Angus Smith and Mr. Dancer estimated that there were 37½ millions of organisms, many of which were recognisable, in 2500 litres of Manchester air.*

Another Method for showing that all the Organisms produced by previously heated Infusions have for their origin the particles which exist suspended in ordinary Atmospheric Air.

Says Pasteur, "I believe it to be rigorously established in the preceding chapters that all the organised productions

* Air and Rain, p. 305.

of infusions previously heated, have no other origin than the solid particles which are always carried in the air and left deposited constantly upon everything. Could there still remain the least doubt of this in the mind of the reader, it will be dissipated by the experiments I will now describe."

The experiments consisted in placing in glass flasks the following liquids, all of which are very changeable in contact with ordinary air, yeast liquor, sugar solution and yeast liquor, urine, beet-root juice, and infusion of pears; the flasks were then drawn out so as to have a long neck with many bends in all directions. The liquid is boiled for some minutes, while the steam escapes plentifully from the open neck; the flasks are then left to themselves without being sealed, and, strange to say, though the air enters, the liquid may be preserved for an indefinite period—an interesting fact for those who are accustomed to make experiments of such a delicate nature as this subject requires. There is no fear of transporting these flasks from place to place, or submitting them to the varying temperature of the seasons; the liquids show not the slightest alteration in taste or smell; they are truly specimens of Appert's food-preserving process. In some cases there was a direct oxidation of the matter, a purely chemical process. But it has already been shown how this action of oxygen was *always limited when organised productions were developed in liquids.* The explanation of these new facts is, that the air on first entering comes in contact with water vapour at the temperature of 100° C., and is so rendered harmless; what follows enters but slowly, and leaves its germs or particles of active matter in the moist curvatures of the tube-neck. After remaining many months in a warm place, the necks of the flasks are cracked off by a file-mark without other disturbance, and in twenty-four hours to thirty-six or forty-eight, fungi and infusoria make their appearance in the usual manner.

The same experiments can be made with milk, but then the milk must be boiled under pressure; milk has been kept for months in these open flasks without change at a temperature of 25° to 30° C. The production of organisms can always be started in these flasks by briskly shaking the liquid or by sealing during ebullition, and after cooling allowing the air to enter suddenly by breaking the point of the tube.

Many such flasks, exhibited at the Academy of Sciences, were preserved with their contents unchanged for eighteen months, although extremely prone to decomposition.

"The great interest of this method is, that it unquestionably proves that the origin of life in infusions which have been boiled is solely due to solid particles suspended in the air. Neither a gas, divers fluids, electricity, magnetism, ozone, things known or hidden causes, there is absolutely nothing in ordinary atmospheric air which, failing these solid particles, can be the cause of the putrefaction or fermentation of the liquids which we have studied." It has so far been definitely proved by Pasteur, and stated in the following manner:—

"1st. That there are constantly, in ordinary air, organised particles which cannot be distinguished from the true germs of the organisms found in infusions.

"2nd. When these particles and the amorphous *débris* associated with them are sown in liquids, which have been previously boiled and which remained unchanged in air previously heated, there appear in these liquids exactly the same forms of life as arise in them when they are exposed to the open air."

"Such being the case, could a partisan of spontaneous generation wish to uphold his principles even in the face of this double proposition? He might, but then his argument would necessarily be of the following kind, of which I leave the reader to judge for himself. There are in the air, he might say, solid particles, such as carbonate of lime, silica, soot, fibres of linen, wool, and cotton, starch granules . . . and besides these organised corpuscles having a perfect resemblance to the spores of the Mucedineæ or the germs of Infusoria. I prefer to attribute the origin of Mucedineæ and Infusoria to the first amorphous substances rather than to the second."

This has actually been asserted. Could there be more eccentric reasoning? Reasoning it is not. That question is beyond the pale of argument, to which common sense dictates the answer.

It is not exactly true that the smallest quantity of ordinary Air gives rise in an Infusion to the Organisms peculiar to this Infusion. Experiments on the Air of various Localities. Inconvenience of employing Mercury in Experiments relative to Spontaneous Generation.

If the smallest quantity of air in contact with an infusion gives rise to organisms, and these organisms are not of spontaneous origin, then it follows that in the minute portion of air there must exist a multitude of the germs of very different organisms; in such numbers, too, that, as Pouchet

says, the air would be so loaded with organic matter as to form a thick fog. Strong as this reasoning is, it would be still stronger if it were shown that different forms of life are derived from different germs: this may be so, but it has not been proved.

Experimental proof of this statement, the error in which lies in gross exaggeration, was made by sealing up during ebullition flasks of 250 c.c. capacity containing about 80 c.c. of various liquids. On breaking the points of these flasks in certain noted places, the air entered with a rush into the empty space, carrying the germs along with it; after re-sealing, the flasks were placed in a warm situation and any change noted. In some cases the decomposition followed, and the production of the usual forms of life; in other cases the flask remained as if they had been filled with heated air, quite unchanged. In two experiments made in the open air after a slight shower in the month of June, both resulted in the production of organisms; in four others, after a heavy rain in the same place, two of the flasks had their contents remain unchanged for at least thirteen months afterwards. These experiments were made, it is easily seen, in an agitated air, but Pasteur carried his labours into the cellars of the Paris Observatory, where the air is quite still except when agitated by the movements of the experimenter, and in that region below the surface of the earth where the temperature is unaffected by the changes of the seasons. It is to be expected that air, in which there is so little to cause its disturbance, would have deposited on the ground the germs which at one time floated in it. A greater proportion of flasks therefore, if opened and re-sealed in such an atmosphere, should have their contents preserved. Out of ten experiments made under such conditions with yeast water, in only one was any living thing found; while eleven experiments made in the court-yard of the observatory at a distance of 50 centimetres from the ground, and at the same time, rendered in every case the usual forms of life; a modification of these trials was made by letting air into flasks of liquid at various mountain heights. Eighty-three flasks, prepared in the manner already mentioned, were experimented on: twenty of these were filled up with air at the foot of the heights which form the first plateau of the Jura; twenty others on one of the peaks of the Jura, 850 metres above the sea-level; and he remaining twenty were carried to Montanvert, near the Mer de Glace, at an elevation of 2000 metres. The result was, that of the twenty opened on the lowest level, eight contained organisms; of the twenty

on the Jura, five only contained any; and lastly, of the twenty filled at Montanvert, while a strong wind blew from the deepest gorges of the Glacier de Bois, one only was altered. The method of opening the flasks was to hold them above the head, with the point turned from the wind, and by a pair of iron forceps, which had just been heated in a spirit-lamp flame, the point was broken. The drawn-out point had been previously scratched with a file and heated ; otherwise particles of dust adhering to the glass would have been carried into the liquid by the in-rush of air.

A remarkable and interesting fact connected with these experiments was, that on one occasion Pasteur opened his flasks, and, on account of not being able to see the flame of his lamp against the brilliancy of the snow, it was impossible to re-seal them ; the flasks were necessarily carried back to the little inn at Montanvert to be closed up. Everyone of these flasks contained organisms after keeping for a short time. On the glacier then, there are no germs in the atmosphere, but at the neighbouring inn the air warms with life, and life from all parts of the world, brought by the travellers. On opening the flasks they were held above the head, so as to prevent the possibility of germs attached to the person being deposited in them.

Explanation of the Cause of Failure of the Experiments in which Mercury is used.

Flasks containing liquids which had been kept for a great length of time were connected with an air-pump and a red-hot platinum tube: after repeated exhaustion and re-filling with heated air, the communication was made between the flask and the platinum tube, and a globule of mercury taken out of a mercury trough in the laboratory, which had previously been introduced into the connecting-tube of india-rubber, was made to roll into the flask ; on re-sealing and keeping for a few days, fermentation ensued in every case, just as certainly as when the asbestos plugs and the adhering germs were sown in similar liquids. This case leaves no doubt regarding the cause of failure of experiments in which the liquid comes in contact with mercury by the flasks being broken under the surface of the quicksilver.

There are other facts which Pasteur established, of great interest and importance in connection with the nutrition of ferments, mucors and vibriones. Instead of experimenting on milk, urine, or solutions containing the liquor from yeast,

he made use of such an infusion as the following; that is to say, a mixture of perfectly definite chemical substances :—

Pure water	100 parts
Sugar-candy	10 ,,
Tartrate of ammonia . .	0·2 to 0·5 part
Ashes of yeast	0·1 part

On impregnating such a liquid, when supplied with heated air, with germs collected from the atmosphere, bacteria, vibriones, and mucors, &c., were soon developed; the albumenoid and fatty matters, the essential oils, and pigments belonging to these organisms being derived from the elements of the ammonia salt, the phosphates, and the sugar. These complete organisms were built up out of the material afforded by such a mixture of simple substances, a fact which is quite contrary to Pouchet's declaration that ovules or germs were evolved from a sort of vitality remaining in lifeless, or, rather, dead, matter—that is to say, matter deprived of life.

A solution consisting of—

Pure water	100 parts
Sugar-candy	10 ,,
Tartrate of ammonia . .	0·2 to 0·5 part
Yeast ashes	0·1 part
Pure calcium carbonate .	3 to 5 parts

showed much the same phenomenon, in fact, differing only by a more marked tendency towards the changes called lactic, viscous, and butyric fermentations; and all ferments, whether vegetable or animal, characteristic of these changes were produced, simultaneously or successively.

Prof. Tyndall, in 1870, gave us a means of investigation, supplementary to the microscope, and of extreme delicacy. Aided by Prof. Huxley, he proved that particles in a liquid, quite invisible under an object-glass readily showing bodies $\frac{1}{100,000}$ of an inch in diameter were revealed with the greatest ease by means of a beam of light. If the air were pure, a beam of sunlight travelling a darkened room would be invisible except where it struck upon the wall. It is the scattering of the light by the floating dust which makes the track luminous, the larger and more numerous the particles the greater the luminosity. Hydrogen, coal-gas, air passed through cotton-wool, and the air of still places, were found to be free from floating matter. The writer, who has devoted much attention to this subject since 1865, made use of this discovery to aid him in a very careful repetition of some experiments published by Dr. Bastian in "Nature" of June

30th, 1870. The following few lines are a slight sketch of the results; for particulars the reader must be referred to the "Proceedings of the Royal Society" for 1872, p. 140.

Tubes cleansed with the greatest possible care, and afterwards heated to redness, were filled with solutions of the same composition as those which it was said by Bastian gave rise to organisms *in vacuo* after heating to so high a temperature as 150° C.; the water and liquids were tested according to Prof. Tyndall's method with a beam of light. After keeping for twelve months, during which time, on frequent examination with a ray of light, no change was seen to have taken place, drops of the liquids were allowed to run on to slips of glass placed in a bell-jar of hydrogen, such being a space shown to be free from floating matter. The microscope, with a higher power than that employed by Dr. Bastian, showed the solutions to be free from all organisms; nevertheless, portions let out into previously heated flasks, in a few days invariably became charged with living things. The original tubes, to which only pure air had been admitted, were kept weeks and weeks, and still no signs of life were visible in them; some of these tubes are in existence now, and still in the same condition. Here, then, were liquids, first, kept *in vacuo*, secondly, in pure air, thirdly, in ordinary air, and only under the last condition did they become filled with life, and that happened in every case. Without wishing to reflect on the work of anyone, it is simply stating a matter of fact to say, that results in favour of the theory of evolution *de novo* may be obtained most easily, and the more careless the experimenter the more successful would he be in that direction. We therefore see not only the extreme caution with which statements advancing heterogenesis should be received, but also the overbalancing weight of evidence contained in well-determined facts tending in an opposite direction.

Printed at the QUARTERLY JOURNAL OF SCIENCE Office, 3, Horse-Shoe Court, Ludgate Hill, E.C.